

We claim:

1. A virally-immortalized hepatocyte, said hepatocyte
  - (a) being derived from a normal liver cell;
  - (b) is nontumorigenic; and
  - 5 (c) naturally produces endogenous therapeutic plasma proteins (TPPs).
2. The hepatocyte according to claim 1, wherein said hepatocyte is derived from a human liver cell.
3. The hepatocyte according to claim 1, wherein said hepatocyte is derived from primary cryopreserved human hepatocytes.
- 10 4. The hepatocyte according to claim 1, wherein said hepatocyte comprises substantially pure simian virus 40 (SV40) DNA.
5. The hepatocyte according to claim 4, wherein said DNA encodes wild type SV40 large T and small t antigens (TAg).
6. The hepatocyte according to claim 5, wherein said SV40 TAg interacts with a  
15 tumor suppressor.
7. The hepatocyte according to claim 6, wherein said tumor suppressor comprises human Rb.
8. The hepatocyte according to claim 6, wherein said tumor suppressor comprises human p53.
- 20 9. The hepatocyte according to claim 1, wherein said hepatocyte has the ability to be maintained in serum free media.
10. The hepatocyte according to claim 9, wherein said serum free media is MCT's proprietary serum free media.
11. The hepatocyte according to claim 10, wherein said MCT's proprietary serum  
25 free media is Multi-Functional Enhancing media (MFE).

12. The liepatocyte according to claim 1, wherein said hepatocyte retains hepatic function.
13. The hepatocyte according to claim 12, wherein said hepatic function is the ability to continue to express hepatic enzymatic activity.
- 5 14. The hepatocyte according to claim 13, wherein said hepatic en2ymatic activity is cytochrome P450 (CYP) enzymatic activity.
15. The hepatocyte according to claim 13, wherein said hepatic enzyme activity of said hepatocyte can be used to assess the effect of chemical entities on the liver.
16. The hepatocyte according to claim 13, wherein said hepatic enzyme activity of  
10 said hepatocyte can be used to assess the effects of drug candidates on the liver.
17. The hepatocyte according to claim 13, wherein said hepatic enzyme activity of said hepatocyte can be used to assess enzyme induction.
18. The hepatocyte according to claim 13, wherein said hepatic enzyme activity of said hepatocyte can be used to assess cellular toxicity.
- 15 19. The hepatocyte according to claim 13, wherein said hepatic enzyme activity of said hepatocyte can be used to assess the effect of the liver on chemical entities.
20. The hepatocyte according to claim 19, wherein said hepatocyte can be used to assess drug metabolism.
21. The hepatocyte according to claim 19, wherein said hepatocyte can be used to  
20 assess species comparisons.
22. The hepatocyte according to claim 12, wherein said hepatic function is the ability to form an acetaminophen conjugate.
23. The hepatocyte of claim 1, wherein said TPPs are selected from the group consisting of albumin,  $\alpha$ -1 antitrypsin, blood clotting factors, transferrin and inter- $\alpha$  -  
25 inhibitor proteins (IaIp).

24. The hepatocyte according to claim 23, wherein said TPPs consist of at least a significant amount of albumin.

25. The hepatocyte according to claim 23, wherein said TPPs consist of at least a significant amount of  $\alpha$ -1 antitrypsin.

5 26. The hepatocyte according to claim 23, wherein said TPPs consist of at least a significant amount of a blood-clotting factor.

27. The hepatocyte according to claim 26, wherein said blood clotting factor is factor VIII or factor IX.

10 28. The hepatocyte according to claim 23, wherein said TPPs consist of a significant amount of transferrin.

29. The hepatocyte according to claim 23, wherein said TPPs consist of at least a significant amount of inter- $\alpha$ -inhibitor proteins (IaIp).

30. The hepatocyte according to claim 1, wherein said hepatocyte can be used to perform a procedure selected from the group consisting of:

- 15 (1) studies of malignant transformation by chemical, physical and viral agents, and transferred genes including oncogenes and high molecular weight genomic DNA from tumors, using standard assays such as anchorage independent growth or tumor formation in athymic nude mice;
- 20 (2) use of cells altered by transfer of oncogenes to screen for potential chemotherapeutic agents;
- (3) studies of cellular biochemistry, including changes in intracellular pH and calcium levels, as correlated with cell growth and action of exogenous agents;

- (4) studies of cellular responses to growth factors and production of growth factors;
- (5) studies of intracellular communication;
- (6) characterization of cell surface antigens;
- 5 (7) cell-cell hybrid studies for identification of tumor suppressor activity;
- (8) identification of novel genes;
- (9) growth of replicating hepatitis virus (as e.g., HBV, HCV, non-A non-13, HAV and other liver-tropic virus, e.g., CMV);
- (10) identification of new drugs to treat hepatitis C virus (HCV) infection;
- 10 (11) expanding of cells for liver transplant and liver function assist devices, both implanted and extracorporeal;
- (12) studies of liver parasites;
- (13) studies of liver diseases;
- (14) identification of potential therapeutic drugs;
- 15 (15) identification of new drug targets;
- (16) identification of chemical and biological agents that induce terminal differentiation;
- (17) studies of the metabolism of carcinogens and other xenobiotics;
- (18) studies of DNA mutagenesis;
- 20 (19) studies of chromosome damaging agents;
- (20) studies of cytotoxicity of drugs, chemical entities, carcinogens, and xenobiotics;
- (21) production of hepatocyte-derived proteins; and
- 25 (22) use of recombinant DNA expression vectors to produce proteins of interest.

31. The hepatocyte according to claim 1, wherein said hepatocyte is Fa2N-4 (ATCC # PTA-5566).
32. The hepatocyte according to claim 1, wherein said hepatocyte is EalC-35 (ATCC # PTA-5565).
- 5 33. A virally-immortalized human primary hepatocyte cell line Fa2N-4 (ATCC # PTA-5566).
34. A virally-immortalized human primary hepatocyte cell line EalC-35 (ATCC # PTA-5565).
35. A method of using the immortalized hepatocyte of claim 1 to assess the effects  
10 of a chemical entity on the liver.
36. The method of claim 35, wherein said chemical entity is a drug candidate.
37. The method of claim 36, wherein said hepatocyte retains hepatic function.
38. The method of claim 37, wherein said hepatic function comprises the ability to express hepatic enzyme activity.
- 15 39. The method of claim 38, wherein said hepatic enzyme activity comprises cytochrome P450 (CYP) enzymatic activity.
40. The method of claim 39, wherein said immortalized hepatocyte is selected from the group consisting of the EalC-35 cell line (ATCC # PTA-5565) and the Fa2N-4 cell line (ATCC # PTA-5566).
- 20 41. A method of using the immortalized hepatocyte of claim 1 to assess enzyme induction,
42. The method of claim 41, wherein said hepatocyte retains hepatic function.
43. The method of claim 42, wherein said hepatic function comprises the ability to express hepatic enzyme activity.

44. The method of claim 43, wherein said hepatic enzyme activity comprises cytochrome P450 (CYP) enzymatic activity.

45. The method of claim 44, wherein said immortalized hepatocyte is selected from the group consisting of the EalC-35 cell line (ATCC # PTA-5565) and the Fa2N-4 cell  
5 line (ATCC # PTA-5566).

46. A method of using the immortalized hepatocyte of claim 1 to assess cellular toxicity.

47. The method of claim 46, wherein said hepatocyte retains hepatic function.

48. The method of claim 47, wherein said hepatic function comprises the ability to  
10 express hepatic enzyme activity.

49. The method of claim 48, wherein said hepatic enzyme activity comprises cytochrome P450 (CYP) enzymatic activity.

50. The method of claim 49, wherein said immortalized hepatocyte is selected from the group consisting of the EalC-35 cell line (ATCC # PTA-5565) and the Fa2N-4 cell  
15 line (ATCC # PTA-5566).

51. A method of using the immortalized hepatocyte of claim 1 to assess the effect of a liver on a chemical entity.

52. The method of claim 51, wherein said hepatocyte retains hepatic function.

53. The method of claim 52, wherein said hepatic function comprises the ability to  
20 express hepatic enzyme activity.

54. The method of claim 53, wherein said hepatic enzyme activity comprises cytochrome P450 (CYP) enzymatic activity.

55. The method of claim 54, wherein said immortalized hepatocyte is selected from the group consisting of the EalC-35 cell line (ATCC # PTA-5565) and the Fa2N-4 cell  
25 line (ATCC # PTA-5566).

56. The method of claim 51, wherein said liver effect on the chemical entity comprises drug metabolism.

57. The method of claim 56, wherein said drug metabolism is measured by the formation of an acetaminophen conjugate.

5 58. A method using the immortalized hepatocytes of claim 1 to perform a procedure selected from the group consisting of:

- 10 (1) studies of malignant transformation by chemical, physical and viral agents, and transferred genes including oncogenes and high molecular weight genomic DNA from tumors, using standard assays such as anchorage independent growth or tumor formation in athymic nude mice;
- (2) use of cells altered by transfer of oncogenes to screen for potential chemotherapeutic agents;
- 15 (3) studies of cellular biochemistry, including changes in intracellular pH and calcium levels, as correlated with cell growth and action of exogenous agents;
- (4) studies of cellular responses to growth factors and production of growth factors;
- (5) studies of intracellular communication;
- 20 (6) characterization of cell surface antigens;
- (7) cell-cell hybrid studies for identification of tumor suppressor activity;
- (8) identification of novel genes;
- (9) growth of replicating hepatitis virus (as e.g., HBV, HCV, non-A non-B, HAV and other liver tropic virus, e.g., CMV);

- (10) identification of new drugs to treat hepatitis C virus (HCV) infection;
- (11) expanding of cells for liver transplant and liver function assist devices, both implanted and extracorporeal;
- (12) studies of liver parasites;
- 5 (13) studies of liver diseases;
- (14) identification of potential therapeutic drugs;
- (15) identification of new drug targets;
- (16) identification of chemical and biological agents that induce terminal differentiation;
- 10 (17) studies of the metabolism of carcinogens and other xenobiotics;
- (18) studies of DNA mutagenesis;
- (19) studies of chromosome damaging agents;
- (20) studies of cytotoxicity of drugs, chemical entities, carcinogens, and xenobiotics;
- 15 (21) production of hepatocyte-derived proteins; and
- (22) use of recombinant DNA expression vectors to produce proteins of interest.